Could thioredoxin h be involved in early response to gravitropic stimulation of poplar stems?

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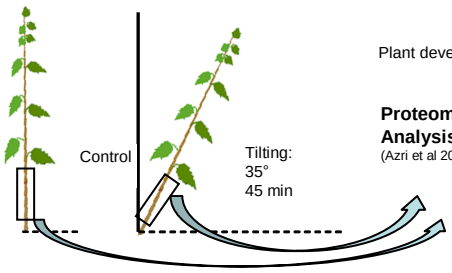
Introduction:

The perception of gravity is essential for plant development. Trees constantly develop specialized woody tissues, termed « reaction wood » to correct inclined branch and stem growth in order to adopt an optimal position. Despite the economical impact of reaction wood occurrence and its importance from a developmental point of view, the perception and response to the gravitational stimulus have not been extensively studied in woody species in which primary and secondary growth occur.

In trees, sedimentable amyloplasts in the endodermal cells may play a role in gravity perception leading to secondary xylem formation, eccentric growth and reaction wood formation in gravi-stimulated tree stems (Nakamura et al., 2001). How the displacement of amyloplasts might trigger a signalling cascade is still a matter of debate. Reactive oxygen species (ROS) are possible second messengers. Redox-dependent regulators are central and flexible mechanisms to control metabolic and developmental activities of the cells. Thioredoxins (Trxs) are 12 kDa proteins that contribute to the redox control by dithiol/disulfide exchange. Thioredoxins *h* were firstly found in the cytoplasm compartment; mitochondria, endoplasmic reticulum and nuclear localization have also been reported (Buchanan and Balmer, 2005). Trxs *h* are encoded by a multigenic family of 8 genes in *Arabidopsis thaliana*, and at least five in *Populus* sp. (Gelhaye et al., 2004). In addition to their role as antioxidant, Trxs *h* was proposed to be involved in modulating redox-dependent signalling cascades (Dietz 2003).

Starting from the observation that many proteins involved in gravitropic response are potential Thioredoxins targets, we investigated the expression and localization of Thioredoxin *h* in the early response to gravitropic stimulation in poplar stems.

Starting points:



Plant developmental stage: 20 developed internodes

Proteomic Analysis
(Azri et al 2009)

- 300 spots were analyzed
- 120 spots showed significant changes after inclination - Mass spectrometry analysis of these spots led to the identification of 60 proteins
- 36/60 proteins (60%) were **potential Thioredoxin targets**

Results:

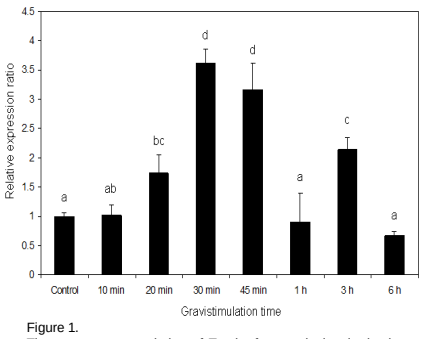
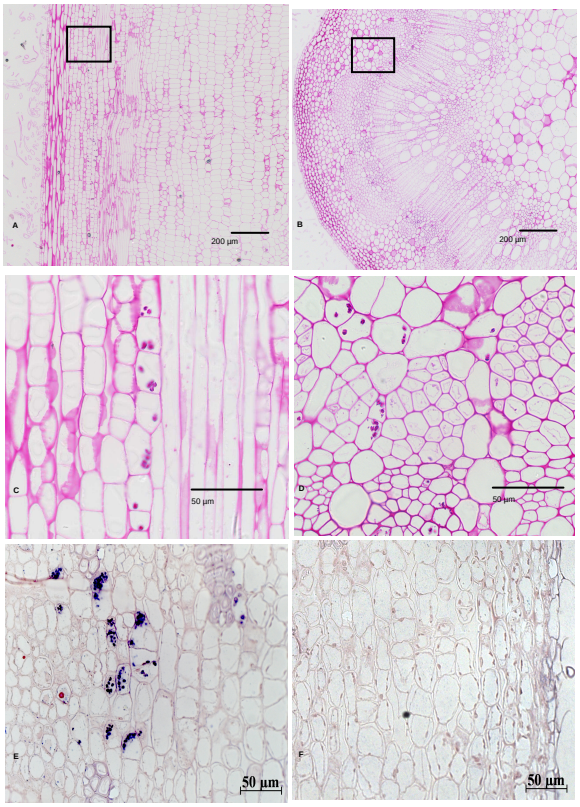


Figure 1.
Time course accumulation of Trx *h* after gravitational stimulus. Total RNAs were extracted from basal internodes of inclined plants for 0 (control), 10, 20, 30, 45 min, 1h, 3h and 6h. The accumulation of relative transcripts was determined by RT-qPCR. Mean values (+SE) of three replicates are shown. Data were analyzed using ANOVA (Stagraphics Plus, version 5.1). Different letters indicate significant differences (P<0.05) for Fisher's LSD pairwise comparisons.

Thioredoxin h expression was induced following gravistimulation

Figure 2.
Sections of poplar stem (4th bottom internode) inclined during 45 min. Longitudinal sections (A, C) or transversal sections (B, D) were stained using Periodic acid/ Schiff (PAS) reaction to detect starch and polysaccharides. Photographs C and D are respectively enlarged views of the photographs A and B (black rectangle area). Transversal sections were probed with the antibody anti-Trx *h*1 (E) or incubated without primary antibody as a negative control (F).

Amyloplasts sedimented at the base of starch sheath cells of poplar stems (Fig. 2A, C). Using *in situ* immunolocalization approach, Trx *h*1 co-localized with the amyloplasts of endoderm cells of the stem (Fig. 2E). A control section incubated without primary antibody (Fig. 2F) attested of the specificity of the signal.



Conclusions and prospect: As a conclusion, our results support an involvement of Thioredoxin *h* (POPTR_0005s25420.1 (Phytozome http://www.phytozome. Net - JGI v2.2)) in the early response to gravitropic stimulus leading to reaction wood formation and poplar stem reorientation: We observed The next step would be to identify the components interacting with amyloplast associated-Thioredoxin in order to dissect the events linked to gravity sensing.